An Improved Two-Step Protocol for Trophoblast Differentiation of Human Pluripotent Stem Cells.

Journal: Curr Protoc Stem Cell Biol

Publication Year: 2019

Authors: Mariko Horii, Tony Bui, Ojeni Touma, Hee Young Cho, Mana M Parast

PubMed link: 31479595

Funding Grants: Bridges to Stem Cell Research Internship Program

Public Summary:
We previously established a two-step protocol for differentiation of human pluripotent stem cells (hPSCs) into trophoblasts, using a StemPro-based minimal medium (EMIM) with bone morphogenetic protein-4 (BMP4). This protocol was suboptimal, resulting in induction of mixed mesoderm and trophoblast markers. Furthermore, adapting hPSCs to StemPro has proven difficult, and prolonged culture in this medium has been shown to promote genomic instability. Therefore, we moved on to the use of new media, including E8, and most recently, StemFlex, for rapid adaptation from feeder to non-feeder conditions. In the new protocol, we have incorporated the WNT inhibitor IWP2 into the first step, resulting in uniform differentiation of hPSCs into cytotrophoblast (CTB)-like cells, without induction of the mesoderm lineage. We also show that, at the end of the second step, there are distinct populations of terminally differentiated multinucleated human chorionic gonadotropin (hCG)-producing syncytiotrophoblast (STB) and HLAG+ extravillous trophoblast (EVT)-like cells.

Scientific Abstract:
We previously established a two-step protocol for differentiation of human pluripotent stem cells (hPSCs) into trophoblasts, using a StemPro-based minimal medium (EMIM) with bone morphogenetic protein-4 (BMP4). This protocol was suboptimal, resulting in induction of mixed mesoderm and trophoblast markers. Furthermore, adapting hPSCs to StemPro has proven difficult, and prolonged culture in this medium has been shown to promote genomic instability. Therefore, we moved on to the use of new media, including E8, and most recently, StemFlex, for rapid adaptation from feeder to non-feeder conditions. In the new protocol, we have incorporated the WNT inhibitor IWP2 into the first step, resulting in uniform differentiation of hPSCs into cytotrophoblast (CTB)-like cells, without induction of the mesoderm lineage. We also show that, at the end of the second step, there are distinct populations of terminally differentiated multinucleated human chorionic gonadotropin (hCG)-producing syncytiotrophoblast (STB) and HLAG(+) extravillous trophoblast (EVT)-like cells. (c) 2019 by John Wiley & Sons, Inc.